

# Detection of Metallothionein in Formalin-Fixed, Paraffin-Embedded Rat Tissue

## Reagent and Antibody Information

[1X Wash Buffer](#)  
[3% Hydrogen Peroxide](#)  
[1% BSA Diluent](#)  
[1X Citrate Buffer](#)  
[DAB Chromogen](#)  
[Hematoxylin](#)

Blocking Solution: Dakocytomation Protein Block Serum-Free Ready-To-Use  
Dakocytomation Corporation  
Carpinteria, CA 93013  
www.dako.com  
1-800-235-5763  
Code No. X0909

Avidin / Biotin Blocking Kit  
Vector Laboratories, Inc.  
Burlingame, CA 94010  
www.vectorlabs.com  
1-800-227-6666  
Catalog # SP-2001

Primary Antibody: Mouse Anti-Metallothionein (E9) Antibody  
Invitrogen  
San Francisco, CA  
www.invitrogen.com  
1-760-603-7200  
Catalog # 18-0133

Negative Control Serum: Purified Mouse IgG1 Isotype Control Serum  
BD Biosciences  
San Jose, CA 95131  
www.bdbiosciences.com  
1-855-236-2772  
Catalog # 557273

Staining Kit: LSAB+ System-HRP  
Dakocytomation Corporation  
Carpinteria, CA 93013  
www.dako.com  
1-800-235-5763  
Code No. K0690

**Note:** This kit includes reagents needed for the secondary antibody (link) and label complex.

## Staining Procedure

Positive Control Tissue: Hair follicles of normal skin  
Stain Localization: Nuclear and cytoplasmic

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.
3. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Microwave

Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer  
(Insert blank slides into any empty slots in the rack to ensure even heating of slides)

Microwave for 5 minutes at power level 5.

Cool for 1 minute. (Add more citrate buffer, if necessary.)

Microwave again for 5 minutes at power level 5. *Temperature Before Cooling Slides* \_\_\_\_\_

Cool 20 minutes at room temperature.

Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.

6. Block with the Dako protein-blocking reagent for 10 minutes at room temperature.

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

DO NOT RINSE THE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

7. Avidin / Biotin Blocking Kit

Lot # \_\_\_\_\_ Exp Date \_\_\_\_\_ New Kit: yes / no

Apply avidin block for 15 minutes at room temperature.

Quick rinse in 1X wash buffer.

Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.  
ONLY WIPE EXCESS BLOCK.

8. Apply the primary antibody at a 1:250 dilution. Incubate for 1 hour at room temperature.

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

For negative control slides, dilute mouse IgG1 control serum so that it's IgG1 protein concentration matches that of the primary antibody (if necessary). Then make a 1:250 dilution. If the

concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

LSAB+ Kit

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

10. Apply the Link (yellow bottle) from the LSAB+ Kit. Incubate for 30 minutes at room temperature.

11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

12. Apply the Label (red bottle) from the LSAB+ Kit. Incubate for 30 minutes at room temperature.

13. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

14. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature.  
(Add 1 drop of DAB per ml of substrate)

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with hematoxylin for 20 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X wash buffer until the tissues turn blue.

19. Dehydrate through the following solutions:

<b>Solutions</b>	<b>Repetitions</b>	<b>Time</b>
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip

*Updated 08/21/06*